REPORT DOCUMENTATION PAGE

Form Approved
OMB NO. 0704-0188

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	12/7/06	final report, 12/15/05 - 9/14/06

4. TITLE AND SUBTITLE
Fiber Optic Detection of Action Potentials in Axons

5. FUNDING NUMBERS
W911NF-06-1-0041

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U. S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSORING / MONITORING AGENCY REPORT NUMBER
49655.1-LS-II

11. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.

12 a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

12 b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

In prior exploratory research, we had designed a fiber optic sensor utilizing a long period Bragg grating for the purpose of detecting action potentials in axons optically, through a change in index of refraction, rather than electrically. The potential application of this technology, long term, is a multi-channel interface to the peripheral nervous system in prosthetic devices. In that prior work, optical signals corresponding to electrically measured action potentials were seen inconsistently because of poor physical contact between the nerve and the optical fiber surface. The main goal of this STIR was to develop a method to improve the nerve/fiber contact, and then to confirm the optical detection approach. A number of mechanical methods for fixing the axon to the fiber were tested, but the buoyancy of the nerve in the electrolyte, as well as its delicacy, were problematic. These challenges have not yet been overcome, but numerous approaches have been ruled out in this research as impractical. The key to future success will be keeping the nerve taut and preventing it from moving.

14. SUBJECT TERMS	15. NUMBER OF PAGES
fiber optic sensor, axon, nerve, action potential, long period Bragg grating	8
	16. PRICE CODE

17. SECURITY CLASSIFICATION OR REPORT	18. SECURITY CLASSIFICATION ON THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	**UNCLASSIFIED**	**UNCLASSIFIED**	**UL**

NSN 7540-01-280-5500

Standard Form 298 (Rev.2-89)
Prescribed by ANSI Std. 239-18
298-102

Enclosure 1

Fiber Optic Detection of Action Potentials in Axons

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Final Report, December 2006

Abstract

In prior exploratory research, we had designed a fiber optic sensor utilizing a long period Bragg grating for the purpose of detecting action potentials in axons optically, through a change in index of refraction, rather than electrically. The potential application of this technology, long term, is a multi-channel interface to the peripheral nervous system in prosthetic devices. In that prior work, optical signals corresponding to electrically measured action potentials were seen inconsistently because of poor physical contact between the nerve and the optical fiber surface. The main goal of this STIR was to develop a method to improve the nerve/fiber contact, and then to confirm the optical detection approach. A number of mechanical methods for fixing the axon to the fiber were tested, but the buoyancy of the nerve in the electrolyte, as well as its delicacy, were problematic. These challenges have not yet been overcome, but numerous approaches have been ruled out in this research as impractical. The key to future success will be keeping the nerve taut and preventing it from moving.

1 Introduction

As discussed in the proposal, the optical signals that were obtained in our prior research were not reproducible. One of the main reasons was that the nerves floated in the crayfish saline solution in which they should remain immersed, and thus did not make good, stable contact with the optical fiber. The peaks in the optical signal associated with action potentials that were obtained previously were taken in air in order to exploit surface tension to hold the nerve onto the fiber. However, this resulted in rapid degradation of the signal. The other issue was that the claw nerve that we used contains a number of axons, and it was not clear whether the electrical and optical signals were coming from the same one.

With this STIR funding, we have taken a step further with this work, exploring, and ultimately rejecting, a number of ways to improve the nerve/fiber contact. Unfortunately, we were unable to hire a dedicated biology post-doc to work for only 6 months, so the work was done by Dr. Smela and a graduate student in her group.

2 Original Set-Up

The aim of the experiments was to show that action potentials could be detected optically. To verify this, electrical and optical signals must be recorded simultaneously. This is a challenge for a number of practical reasons. First, the optical fiber with the long period Bragg grating (LPG) is very delicate because the plastic jacket has been stripped from the fiber in order to write the grating. The stripped region cannot be bent at sharp angles or otherwise stressed, or the fiber breaks. In our previous work, a fiber-holder had been constructed to support the fiber and hold it taut (Figure 1). Second, the nerve needs to span two pairs of electrodes for the electrical measurements. The electrodes need to run perpendicular to the optical fiber, and they must be electrochemically stable upon repeated application of voltages in saline solution. The electrode

wires were therefore placed over the optical fiber and glued onto the fiber holder substrate. Lastly, the nerve needs to be placed over both pairs of electrodes and in stable contact along the length of the LPG, and immersed in crayfish saline solution. Thus, the fiber holder had a well to contain the electrolyte.

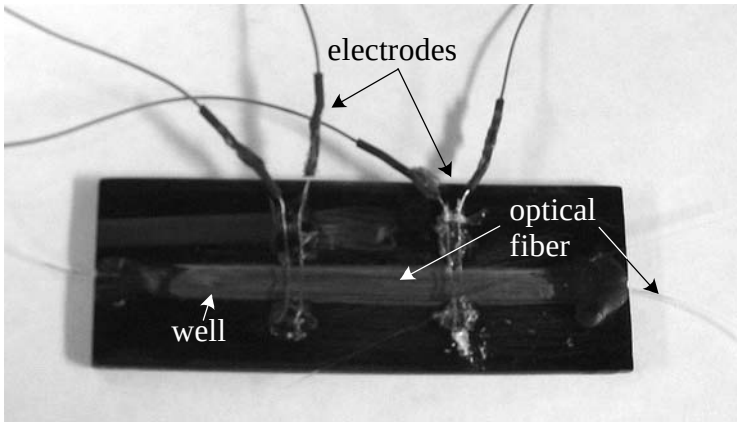

Figure 1. Previously used holder for the optical fiber and the electrode wires.

3 New Nerve Preparation

Prior work had utilized the claw nerve and the abdominal nerve (the nerve in the tail); the latter was more difficult to explant without damage. An alternative method of preparing the abdominal nerve explant was learned from Prof. Jens Heberholtz in the Biology department at the University of Maryland The abdominal nerve is attractive for this research because it has two large axons, the medial giant (MG) and lateral giant (LG), along both sides, near the top (Figure 2). The lateral giants can be stimulated individually, since they are the largest and therefore fire at the lowest applied voltage: by starting at a low voltage and increasing it gradually, the first signal to be seen electrically comes from these. The other axons don't begin to fire until higher voltages are applied, which can also be observed on an oscilloscope. The abdominal nerve thus allows relatively straightforward manipulation due to its large size combined with only single axon firing.

The abdominal nerve lies just below the soft shell on the ventral side of the abdomen (the "belly" side of the crayfish tail). The preparation involves cutting the shell along the sides and the back of the tail, removing it, and then picking out all the muscle above the nerve with tweezers. This process takes at least 30 minutes, which is a drawback since the axon degrades in the meantime.

Once the muscle has been removed, the nerve is exposed on all sides and can be stimulated electrically while still in the shell. Electrical stimulation is performed with one pair of electrodes, and detection is done with a second pair some distance away. The electrodes consist of a pair of fine Teflon-coated Ag wires fixed inside a glass capillary. Micromanipulators into which the capillaries are clamped allow the wires to be positioned in x, y, and z.

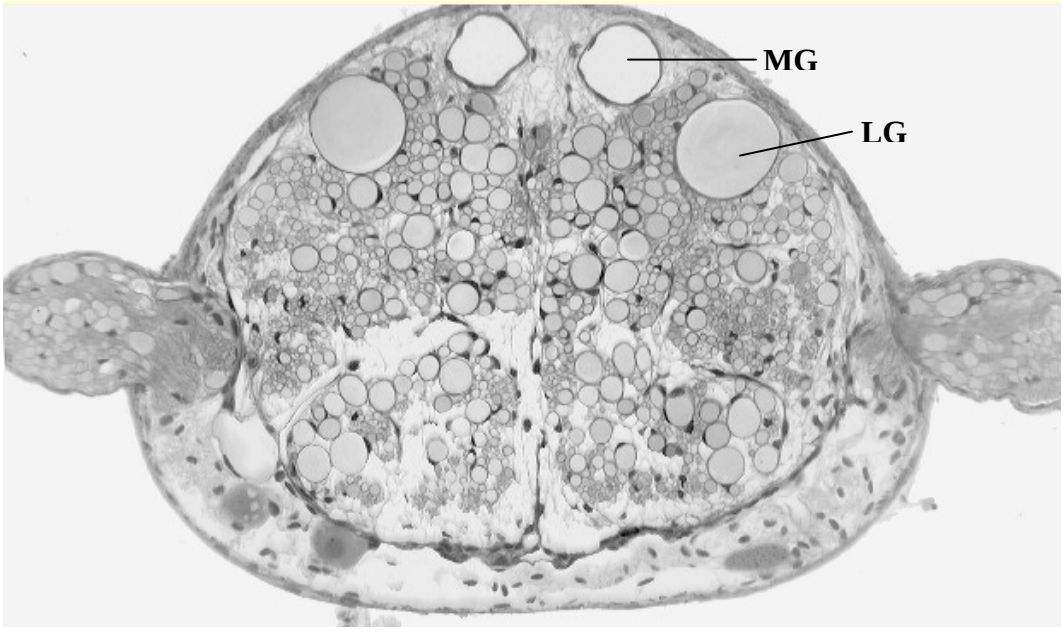

Figure 2. Cross section of the crayfish abdominal nerve from B. Mulloney and W. Hall, UC Davis, http://www.science.smith.edu/departments/NeuroSci/courses/bio330/labs/LAanatomy/transverse.html. The medial and lateral giant axons are labeled. Two roots (see Figure 3) can be seen on either side.

Because of their immersion in saline solution, the receiving electrode pair picks up an electrical signal traveling through the solution, called the artifact. This has the same shape as an action potential, and a comparable time of arrival. To tell it apart from a spike traveling through the nerve, one needs to adjust the voltage: the artifact changes size commensurately, but the action potential does not. To separate the two signals in time, the electrodes should be as far apart as possible.

A drawback to this approach of contacting the nerve is that the wires, while only 0.005" = 125 μm in diameter, are nevertheless significant in size compared to the nerve, which is several hundred μm in diameter, and the wires are much stiffer. Without close mechanical contact, the electrical contact is poor, but pressing on the nerve with the wires can cause damage.

The supplies and manipulators necessary for this work were purchased for our laboratory. The electrodes were fabricated, and the techniques were practiced so that we could reproduce this preparation. The most important of these supplies were tweezers with a special grip for holding onto slippery biological tissue to be used during the dissection.

4 Approach 1: Pin Axon Down over Fiber

Since the fiber holder used previously did not allow for a means of fastening down the axon, and protein coatings such as polylysine to improve adhesion had proven ineffective, the research focused on mechanically fastening the nerve in place. After removing the abdominal nerve, either using the new back-of-the-shell preparation or a faster and more direct approach of removing the soft shell from the ventral part of the abdomen, the nerve was pinned to a polydimethylsiloxane (PDMS) base inside a Petri dish. This required that the nerve be removed

in such a manner that the ganglia branching off of it (Figure 3) be kept as long as possible. Pins placed into the nerve itself damage the axons, so the pins were placed into the ganglia instead.

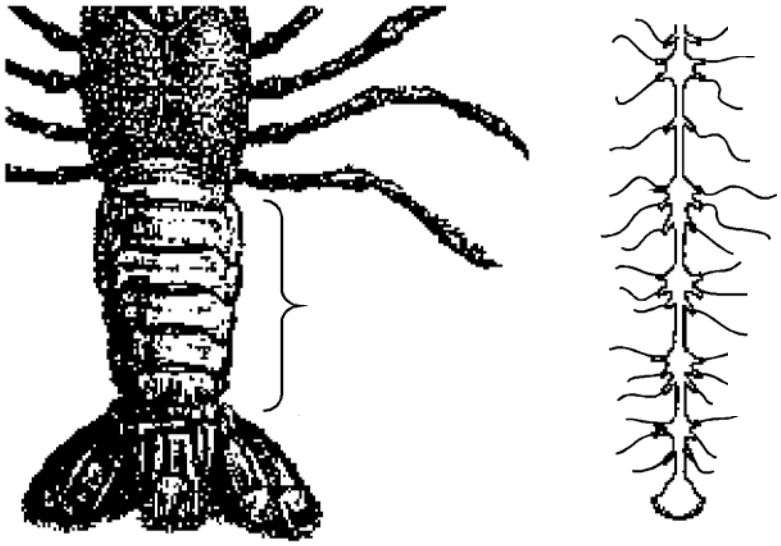

Figure 3. Crayfish tail viewed from the top (back) side and the abdominal nerve chord that runs along the tail section. Image taken from http://www.science.smith.edu/departments/NeuroSci/courses/bio330/labs/L7cns.html.

For the work described here, "dummy" optical fibers were used, which were glass optical fibers with the protective plastic jackets stripped off of a 2-3" section to mimic the LPG fibers. This renders the fibers brittle, and care must be taken in handling them.

Given the long preparation time, many of the experiments were conducted by, after decapitation, removing the soft shell on the ventral side of the abdomen, which exposes the nerve directly. This was done by peeling the shell back gradually and cutting the dorsal roots that are attached to the shell, trying to keep them as long as possible.

4.1 Tying the Nerve to the Fiber

Prof. Heberholtz had suggesting tying off the two ends of the nerve to keep it active longer. Using a hook tool and fine thread, this was done with the nerve still in the shell. The dummy optical fiber was laid alongside the nerve, and on one end, using the same thread, the nerve was then tied to the fiber. On the other end, the nerve was ligated and tied to the fiber with a single loop. Unfortunately, the nerve could not be kept taut since the thread slipped readily on the optical fiber: the nerve therefore "sagged" in the center and did not contact the area where the grating would be (Figure 4).

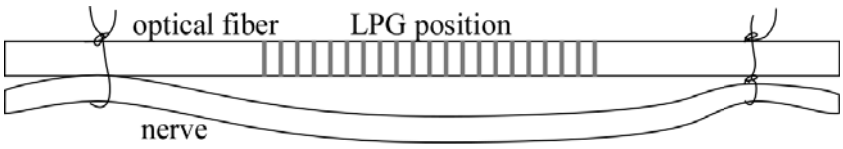

Figure 4. Tying the nerve to the fiber is unsuccessful because the thread slides along the optical fiber, so the nerve cannot be held straight against it.

Using the Ag electrodes and the x-y-z positioners, electrical contact was made to the nerve, and stimulation at 9 V caused the connected muscles to twitch. Action potentials were recorded with the second electrode pair, but not consistently. One issue with the Ag wires is oxidation at these voltages: the Teflon coating is stripped from the ends of the wires, and the exposed parts turn black. Another issue is that the electrode pair develops a capacitance in a rather short time, most probably from salt accumulating in the glue plug at the end of the capillary that holds the wires in place. Thus, fresh electrodes should be prepared prior to each experiment.

4.2 Fixing the Optical Fiber

Based on these results, it was decided that the nerve should be pinned down over the optical fiber. PDMS is commonly used as a base for pinning during dissections. PDMS is an elastomeric, biocompatible, transparent polymer that is formed by mixing two precursor solutions together and curing.

First, the optical fiber needed to be fixed in place on the PDMS. A narrow groove was cut into a PDMS-filled petri dish into which the optical fiber could be fixed. However, this required that the optical fiber make a rather sharp turn at the edges of the dish (Figure 5), which put a stress on the fairly stiff fiber, causing it to pop out of the groove; it cannot be pushed too deeply down, or the grating would not be exposed. This stress can also cause the fiber to break. Finally, this mounting method imparted a curvature to the grating part of the fiber, which should be keep straight.

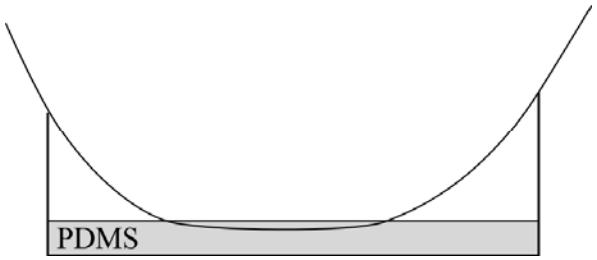

Figure 5. Optical fiber fixed into a Petri dish by embedding in PDMS.

Another method of fixing the optical fiber in place was tested that involved laying the optical fiber flat over a slab of PDMS, constructing concentric ring-shaped walls, and filling the gap with uncured PDMS solution to harden in place, but the mixture is too fluid, and it leaked out and covered the fiber.

The PDMS was therefore replaced by Loctite 3108, a UV-curable elastomer. The uncured precursor is less fluid, having a consistency similar to that of toothpaste so that it can hold its shape. The precursor was filled to the top of a petri dish, covered with plastic wrap, and cured under a UV lamp. The heat generated caused the plastic wrap to wrinkle, so that the surface of

the Loctite was not perfectly smooth. The fiber was laid parallel to a shallow wrinkle. Both ends were covered with a knob of additional Loctite, and this was cured. This configuration held the optical fiber firmly in place without stress. The polymer and fiber were rinsed with acetone and then soaked for several days in several changes of deionized water to remove any water-soluble monomers that might be toxic to the nerve.

4.3 Pinning the Nerve over the Optical Fiber

A nerve was cut out of the shell and placed into the Loctite surface, which was fixed to the bottom of a larger petri dish filled with crayfish saline. The nerve was then fixed over the fiber by pinning the roots. This pinning process took considerable time because of the buoyancy of the nerve and its delicate, jelly-like consistency, which is easily damaged by pins and tweezers. Unfortunately, once pinned, the nerve still floated in the solution, without making good contact with the optical fiber (Figure 6). Furthermore, when the Ag wires were brought into contact with the nerve, they pushed it off of the fiber.

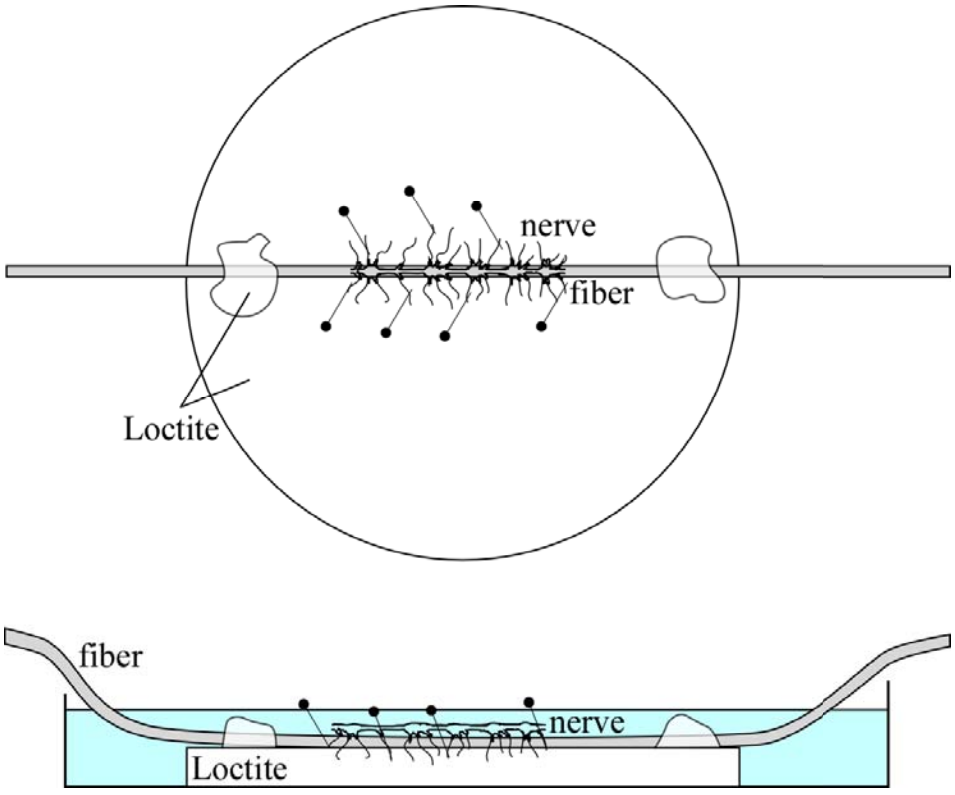

Figure 6. Overhead and side views of a nerve pinned down over an optical fiber fixed to an elastomeric substrate.

Another Loctite substrate was prepared having a ridge under the optical fiber with the hope that the roots could be pulled downward upon pinning. This configuration was some improvement. Another issue that must be considered is that once explanted, the nerve coils and tangles, and it becomes difficult to keep straight and at the proper rotation.

5 Approach 2: Leave Axon in Shell

5.1 Front-Side Approach

In order to keep the nerve taut and at the right rotation, the next approach involved leaving it in the shell and bringing the fiber alongside it. The shell was removed from the ventral side of the abdomen. The activity of the nerve was verified by electrical stimulation, which produced muscle twitches. A dummy fiber was then manipulated to try to bring it into contact with the nerve. The following issues arose. First, the clear, colorless fiber became virtually invisible, and it was particularly difficult to discern the region from which the jacket was stripped, where the LPG would have been. Second, the nerve is still not held in place tautly enough, and moves when touched by the fiber because the front-side preparation involves cutting some of the roots (the ones that are attached to the soft shell). Third, the dish holding the saline needs to be more shallow in order to avoid too large a curvature on the fiber. Fourth, it is difficult to maneuver the fiber into position, and to do so without breaking it.

5.2 Back-Side Approach

This technique was thus attempted using the Heberholtz preparation and a dish with lower sidewalls. The thorax was left on so that the nerve would not be severed, with the hope that this would hold it in a more fixed position. However, to remove the muscle tissue, some of the roots had to be severed. On the plus side, electrode contact was straightforward, and the nerve remained active, as indicated by muscle movement. The thorax and tail fan were, unfortunately, too high above the nerve, which was at the bottom of the shell, to be able to bring the optical fiber into position. The nerve ends were therefore ligated and the thorax and tail fan removed. This left the nerve rather loose, being connected only to the soft shell. The ligating thread was therefore pinned down to the PDMS at the bottom of the dish. The edges of the part of the fiber with the jacket stripped off were marked in black permanent ink, so they could be easily seen. It once again proved difficult to maneuver the optical fiber, and additional manipulators would be needed to make this work. Given the space constraints, it would not be straightforward to fit everything closely enough.

At the end of this experiment, the nerve was cut from the shell to try to attach it to the optical fiber using surface tension, as had been done previously. The nerve immediately attached itself along the thread, however, where it stuck permanently. It is not clear whether this is due to the roughness of the thread, or to its composition.

5.3 Cutting open the Nerve

The nerve is covered with a tough sheath. It is an attractive idea to cut the nerve open so that direct contact could be made between the surface of the optical fiber and the large axons. Previously, we have tried to dissolve the sheath, for example using protease, or to combine protease digestion of the sheath with mechanical removal. This does not produce a satisfactory result, just damaging the nerve. An ultra-fine, ultra-sharp dissection tool was purchased with the plan of bisecting the nerve. However, the sheath is too tough to be cleanly cut; rather, it tears.

6 Summary of Lessons Learned

The buoyancy of the nerve and its delicacy pose problems in bringing the nerve parallel to, and in direct physical contact with, the optical fiber. These challenges have not yet been overcome, but numerous approaches have been ruled out in this research as impractical. The key to success will be keeping the nerve taut and preventing it from moving.

If Ag wires mounted in capillaries are used with micromanipulators to make contact with the nerve, fresh electrodes must be prepared for that day's work, and care must be taken not to damage the nerve with too much pressure.

7 Future Work

Future experiments in the short term will focus on the use of surgical glue, which is biocompatible and can be used in water. This is based on the successful use of the photo-curable glue to fix the optical fiber, which was quite effective and held it in place so that it could not readily be broken. The part of the fiber outside the glued areas was covered by the jacket, and could therefore be manipulated without breaking the fiber. The pins allowed too much vertical movement of the nerve. The plan is to glue down one side of the nerve, then use a pin to stretch the nerve and hold it in position while a second spot of glue is used to fix the other end in place. This will be tried both in a petri dish, which has the drawback that it is difficult to orient the fiber rotation angle vis-à-vis the optical fiber, and in the shell as described at the end of section 5.2, which has the drawback that it may be difficult to re-use the LPG, which is fairly expensive.

In terms of electrical contacts to the nerve, there is the possibility of metalizing the fibers and then patterning the metal film to form flat electrodes directly on the fiber, thus avoiding the need to introduce wires that are almost as large as the nerve itself. This patterning would be possible using a maskless lithography system from Intelligent Micropatterning that the Fablab at the University of Maryland is considering purchasing. This system has a z-stage that allows for patterning on curved surfaces. The metal patterning would need to occur prior to writing the grating

In the long term, once the optical sensing of action potentials is established, axons will need to be regenerated over the LPG by coating the fiber with nerve growth factor and other proteins. This requires a biologist such as Lnenicka at SUNY in Albany with expertise in the regeneration of axons.